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TITLE: Treatment of Traumatic Brain Injury by Localilzed Application of Sub-atmospheric Pressure to the Site of Cortical Impact

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14. ABSTRACT

Each major war tends to have a ósignature injuryô, with traumatic brain injury (TBI) associated with the Iraq wars (Operation Iraqi Freedom II and Operation Enduring Freedom) due to the high incidence of personnel injured by IED (improvised explosive devices). The large gyrencephalic brain of swine is similar to humans, thus a swine model of a localized TBI treated by the controlled application of sub-atmospheric pressure was employed. Work in Year 1 showed that application of 100 mm Hg to the site of the TBI resulted in a significantly ($p < 0.01$) smaller mean contused brain tissue volume and mean intra-cranial hemorrhage volume than that seen in control (non-treated) or those injuries treated with 50 mm Hg. Work in Year 2 showed that application of 100 mm Hg to the site of the CCI for 5 days was necessary to prevent a late increase in intracranial pressure (ICP). In Year 3, work completed showed that that a 3 hour delay between injury and initiation of vacuum treatment was as efficacious as immediate treatment, and that a 6 hour delay was slightly less efficacious. The results of this swine model of a focal TBI demonstrate that a 5 day application of 100 mm Hg vacuum to the site of injury greatly diminished the severity of the injury, even with delays in application of up to 6 hours post injury.

15. SUBJECT TERMS

Traumatic brain injury, sub-atmospheric pressure treatment, vacuum, mechanical tissue resuscitation

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Introduction

Each major war tends to have a 'signature injury', with traumatic brain injury (TBI) associated with the Iraq war (Operation Iraqi Freedom II and Operation Enduring Freedom) due to the high incidence of personnel injured by IED (improvised explosive devices). Our previous study in a rat model showed that Mechanical Tissue Resuscitation (MTR – the controlled application of vacuum) to the cerebral cortex following a controlled cortical impact (CCI) injury reduces brain edema and the extent of injury, modulates metabolites in injured neuronal tissues, preserves neuronal tissue, and improves functional recovery. The large gyrencephalic brain of swine is similar to humans, thus a swine model of CCI injury and MTR treatment was evaluated for future human clinical applications. Year 1 goals were to determine the level of vacuum which resulted in the most efficacious result. Year 2 goals were to determine the length of time that the 100 mm Hg of applied sub-atmospheric pressure (determined in Year 1) was necessary to prevent late cell injury and death and late increase in intracranial pressure. Year 3 goals were to determine the length of delay between creation of the injury and application of the localized vacuum to the site of injury to prevent or minimize the size and progression of the injury.

Body

Year 1 goals stated in the Statement of Work included determination of the preferred level of sub-atmospheric pressure which results in development of the least significant injury. Post-injury, animals were treated with either 50 mm Hg or 100 mm Hg vacuum. 100 mm Hg was determined to be more efficacious.

Year 2 goals stated in the Statement of Work included determination of the length of time for application of sub-atmospheric pressure necessary to prevent cell death and injury due to secondary injury, and also to prevent a late increase in intracranial pressure. Animals were treated for 3 or 5 days with application of 100 mm Hg vacuum to the site of the focal trauma (level of sub-atmospheric pressure determined in Year 1). 5 days treatment was determined to be more efficacious.

A new MRI machine was installed during Year 3 which resulted in a request for a non-funded extension. The old machine was removed and the new one installed and calibrated, resulting in a several month delay. Data analysis is still being completed to compare image analysis from the old machine to images captured by the new machine.

Year 3 goals stated in the Statement of Work included determination of the length of time between creation of a focal TBI and then efficacious application of vacuum application to the site of the focal injury. Injuries were created and animals were treated with 100 mm Hg vacuum for 5 days, with treatment beginning immediately (based on Year 1 and 2 studies), or after a 3 or 6 hour delay post injury. Application of vacuum 3 hours post injury was equivalent to immediate application. A 6 hour delay resulted in a lesser, but still efficacious response.

Year 1.

Thirty four (34) female domestic swine (22-33 kg) were procured and randomly divided into groups: operated sham; CCI non-treated; CCI MTR treated - 50 mm Hg; or CCI MTR - 100 mm Hg. For creation of the CCI, animals were anesthetized and a 17 mm diameter craniotomy was performed over the right front parietal cortex. A pneumatic impactor pistol was used with the plunger parameters of 12 mm diameter, 12 mm in depth, 2.7m/s velocity, and 250ms dwell time. For MTR treatment, a sterile vacuum dressing was placed in the bony defect and either 50 mm Hg or 100 mm Hg was applied continuously for 72 hours. 72 hours post surgery, all animals were analyzed by MRI (GE Signa EchoSpeed 1.5-T scanner). Parameters analyzed included: MR sagittal T1 imaging; coronal T2 imaging; coronal MPGR (Multi-Planer Gradient Echo); Axial T2* Contrast Enhanced Perfusion (0.2 ml/Kg Magnevist contrast by power injection). All animals were euthanized and perfused with 4% para-formaldehyde through the ascending aorta 8 days post-injury. The brain was removed and postfixed in the same fixative solution overnight at 4ºC. After a PBS rinse, the brains were placed in 30% sucrose at 4°C before they were snap-frozen in O.C.T and stored at -80ºC. Coronal sections of the injured area were cut into 20 µm thick sections using a cryostat, mounted, and kept frozen until use. Sections were collected every 0.5 mm through all injured area over a total distance of 2 cm. Sections were examined after staining with haematoxylin and eosin (H&E). (Figure 1)

Total contusion injured brain volumes were measured in all coronal MR T2 weighted images as the sum of all injury areas in both groups. (Figure 2) The injured area was identified and traced as a hyperintense region ipsilateral to the injured site. There was a large area of T2 hyperintensity (edema) sometimes associated with hypointensity (hemorrhage) and herniation in T2-weighted images.

The mean contused brain tissue volume is 6.59±1.76 cm^3 in non-treated injured animals. For animals treated with MTR 100mmHg, the contused brain tissue volume decreased to 3.44±1.14 cm^3. For animals treated with MTR 50 mm Hg, the contused brain tissue volume increased to 9.49 ±3.71 cm^3. Statistical analysis showed a significant difference between injured non-treated and injured with MTR 100 mm Hg treatment ($p < 0.01$). The MTR 100 mm Hg injured brain volume was also significantly smaller than that for the MTR 55 mm Hg animals ($p < 0.01$). There is no significant difference between the MTR 50 mm Hg group compared to the non-treated injured group.

The mean hemorrhage volume in non-treated animals (375.75±348.9$mm^{3)}$ is significantly ($p < 0.01$) larger than the mean hemorrhage volume in injured MTR 100mmHg treatment (53.31±67.81 mm^3). The MTR 100 mm Hg mean hemorrhage volume is significantly ($p < 0.01$) smaller than the mean hemorrhage volume for MTR 50 mmHg (606.84±364.05 mm^3). There was no statistical difference between the mean hemorrhage volume of the non-treated injured and the MTR 50 mm Hg group.

At 8 days after injury, histopathologic results demonstrated major neuronal tissue loss and intracerebral hemorrhage in non-treated injured brains (Fig. 1 left), which confirmed that hypointense lesions seen on T2-weighted and gradient echo MR images were hemosiderin deposits of hemorrhages after injury. Less neuronal loss and hemorrhage in the injured area were observed after MTR treatment (Fig 1 right).

Figure 1. Left. Injured, non-treated brain slices 8 days post injury. Right. Injured, MTR (100 mm Hg, 72 hour treatment) treated brain slices 8 days post injury. Slices are 3 mm apart through the center of CCI site. H&E. Original magnification 2X.

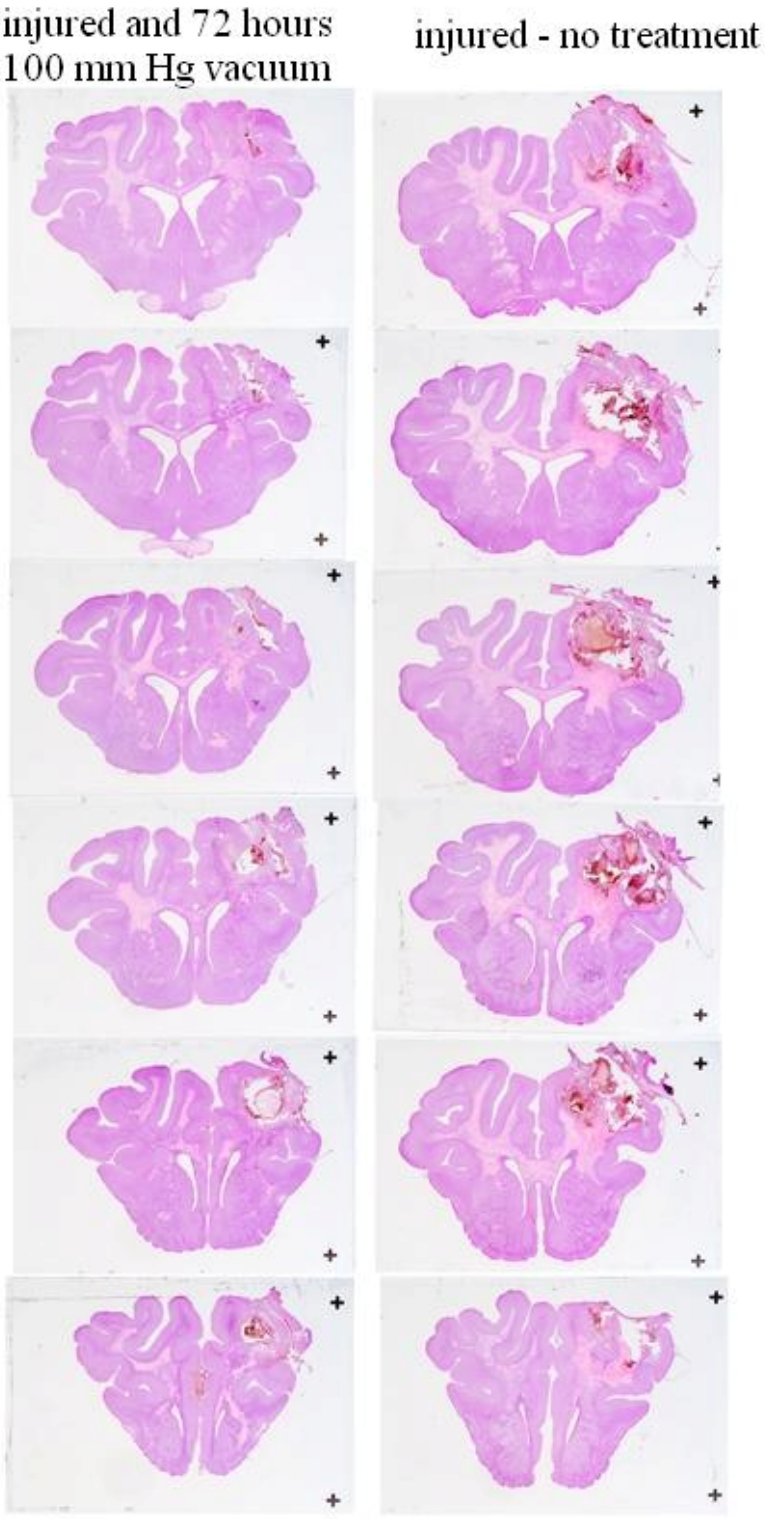

Figure 2. The mean total brain tissue injury volumes measured in T2-weighted MR images in traumatic brain injury pigs with/out MTR treatments. The MTR 100 mm Hg group was significantly ($p < 0.01$) smaller than the non-treated control and MTR 50 mm Hg groups. There was no significant difference between the MTR 50 mm Hg and the non-treated control group.

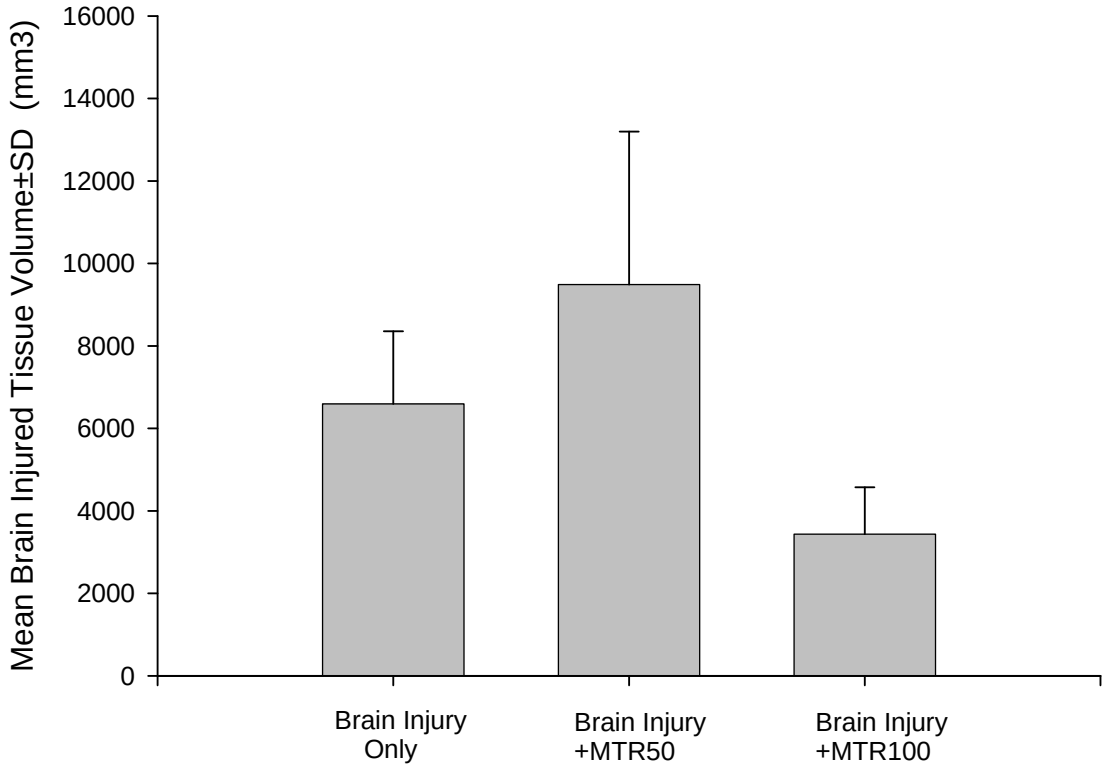

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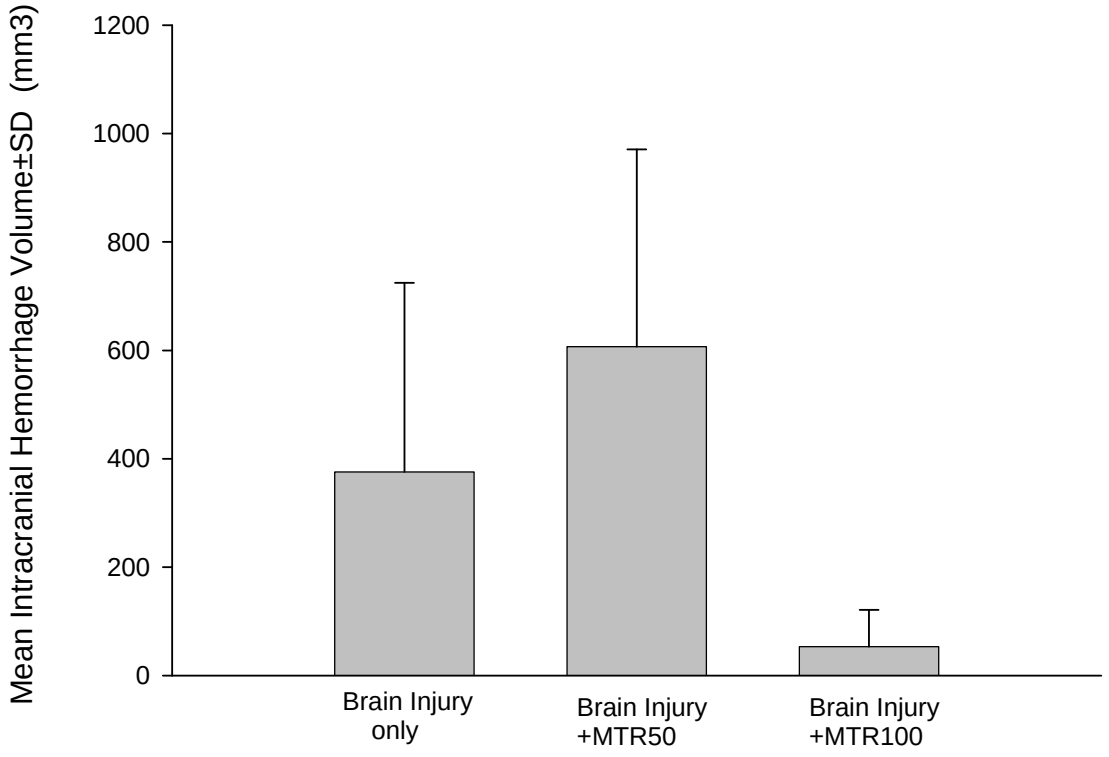
Figure 3. The mean intracranial hemorrhage volumes measured in gradient echo MR images in pig traumatic brain injury with/out MTR treatments. The MTR 100 mm Hg group was significantly ($p < 0.01$) smaller than the non-treated control and MTR 50 mm Hg groups. There was no significant difference between the MTR 50 mm Hg and the non-treated control group.



Year 2.

Thirty four (34) female domestic swine (22-33 kg) were procured. For MTR treatment, a sterile vacuum dressing was placed in the bony defect and 100 mm Hg was applied continuously for 3 or 5 days. Intracranial pressure was monitored by telemetry. Five days post surgery, all animals were analyzed by MRI (GE Signa EchoSpeed 1.5-T scanner). Parameters analyzed included: MR sagittal T1 imaging; coronal T2 imaging; coronal MPGR (Multi-Planer Gradient Echo); Axial T2* Contrast Enhanced Perfusion (0.2 ml/Kg Magnevist contrast by power injection); and metabolic spectral analysis. Surviving animals were euthanized 10 days post injury and perfused with 4% para-formaldehyde through the ascending aorta 8 days post-injury. The brain was removed and postfixed in the same fixative solution overnight at 4ºC. After a PBS rinse, the brains were placed in 30% sucrose at 4°C before they were snap-frozen in O.C.T and stored at -80ºC. Coronal sections of the injured area were cut into 20 µm thick sections using a cryostat, mounted, and kept frozen until use. Sections were collected every 0.5 mm through all injured area over a total distance of 2 cm. Histological staining and analysis is being completed.

All 10 animals in the 5 day treatment group survived (100% survival) with one suffering a seizure. Three of the eight animals (37.5%) died after the vacuum was discontinued on Day 3 but prior to day 10 post injury. (Figure 4) An additional two animals (out of the 5 surviving) exhibited signs of ataxia and one of these animals had difficulty eating. Thus 5 of 8 animals had significant morbidity or mortality. The 3 day treatment was thus discontinued. The 1 day treatment was not performed due to the high morbidity and mortality rate in the 3 day treatment group.

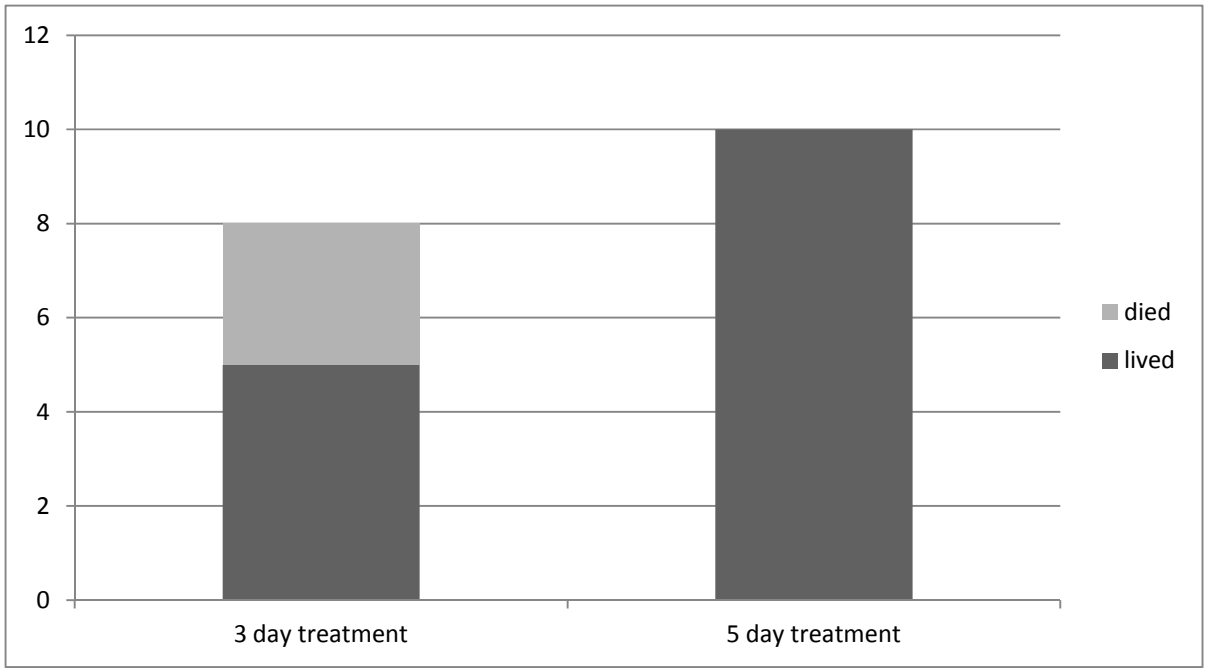


Figure 4. Bar graph showing survival rates for animals in either the 3 day treatment group (50% survival) or the 5 day treatment group (100% survival).

The volume of cerebral hemorrhage for animals treated for 5 days (146 $\pm$ 71 mm3 - SPS dressing and 166.2 $\pm$ 67 mm3 - WS dressing) was significantly less ($p < 0.2$) than for animals in then on-treated control group (564 $\pm$ 112 mm3). (Mean $\pm$ SEM) Figure 5.

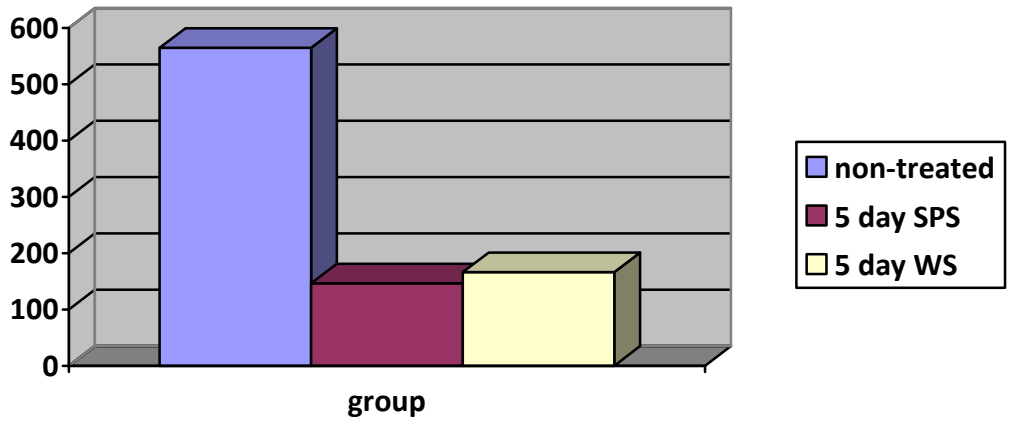


Figure 5. Hemorrhage volume at site of injury as measured from MRI. Treated groups had significantly less hemorrhage volume than non-treated group.

T2 weighted MRI's of the animals treated for 5 days showed a significant decrease in water content (swelling) in the area of injury. Also evident from the images is the herniation of the brain through the craniotomy for the non-treated group. No herniation is evident for the animals treated for 5 days. Two different vacuum dressings were employed (labeled SPS and WS in Figure 6 below) with equal efficacy.

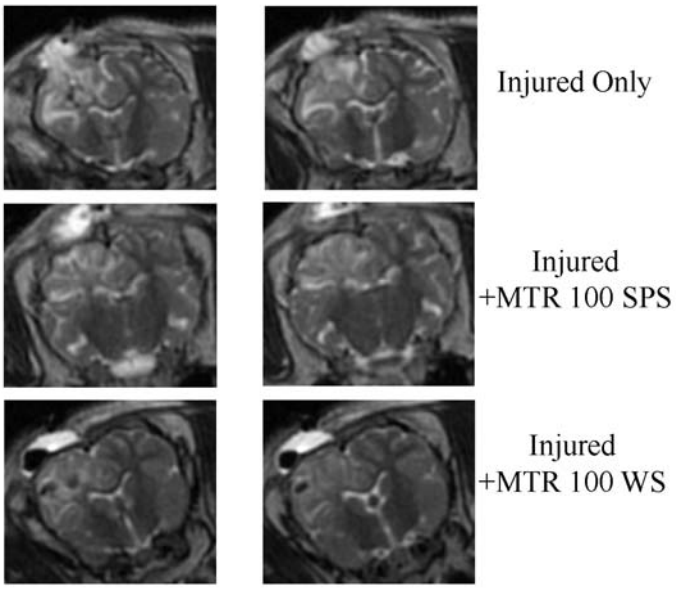


Figure 6. T2 weighted MRI of non-treated and 5 day treatment animals. Injured site is on left side of brain. Herniation of the brain through the craniotomy is visible in the non-treated image (top). Two different vacuum dressing were employed (SPS (middle) and WS (bottom)) with equal efficacy.

Metabolite	sham	Injured, no treatment	Injured with 100 mm Hg vacuum treatment
Creatine	5.06±0.58	2.81±2.52	4.71±0.44
Glutamine	1.99±2.76	0±0	3.01±5.22
Glutamate	7.86±2.74	5.05±4.73	4.83±4.70
Myoinositol	6.33±1.14	8.18±8.02	4.65±2.87
Lactate	0.72±1.25	6.30±7.56	2.69±2.05
NAA	6.97±1.15	2.41±2.30	5.01±1.80
NAAG	1.64±1.13	1.60±2.47	0.64±1.27
Taurine	0±0	1.53±2.92	0.75±1.48
GPC	2.00±0.32	1.23±1.20	1.03±0.96
PCh	0±0	0.14±0.41	0.60±0.82
Guanidoacetate	1.37±1.35	14.63±21.92	1.06±2.38

Table 1. Metabolic spectral scan of excitatory amino acids and related mediators, comparing levels in control (sham) animals, injured but non-treated animals, and animals injured and treated with 100 mm Hg vacuum for 72 hours. NAA = N-acetyl aspartate, GPC = glycerylo-phosphocholine, PCh = phosphorylated choline

After three days of vacuum (applied immediately post injury), levels of the majority of mediators examined in treated animals approached levels in the non-injured control animals for the majority of factors examined, while injured non-treated levels did not.

Year 3.

A total of thirty two (32) female domestic swine (22-33 kg) were procured (including those treated immediately (0 hour delay) in previous years). For MTR treatment, a sterile vacuum dressing was placed in the bony defect and 100 mm Hg was applied continuously either immediately post injury, or after either a 3 or 6 hour delay. Vacuum was applied for a total of 5 days. Intracranial pressure was monitored by telemetry up until the time of MR imaging. Five days post surgery, all animals were analyzed by MRI (GE Signa EchoSpeed 1.5-T scanner). Parameters analyzed included: MR sagittal T1 imaging; coronal T2 imaging; coronal MPGR (Multi-Planer Gradient Echo); Axial T2* Contrast Enhanced Perfusion (0.2 ml/Kg Magnevist contrast by power injection). All animals were euthanized 10 days post injury and perfused with 4% para-formaldehyde through the ascending aorta 8 days post-injury. The brain was removed and postfixed in the same fixative solution overnight at 4ºC. After a PBS rinse, the brains were placed in 30% sucrose at 4°C before they were snap-frozen in O.C.T and stored at -80ºC. Coronal sections of the injured area were cut into 20 μm thick sections using a cryostat, mounted, and kept frozen until use. Sections were collected every 0.5 mm through all injured area over a total distance of 2 cm.

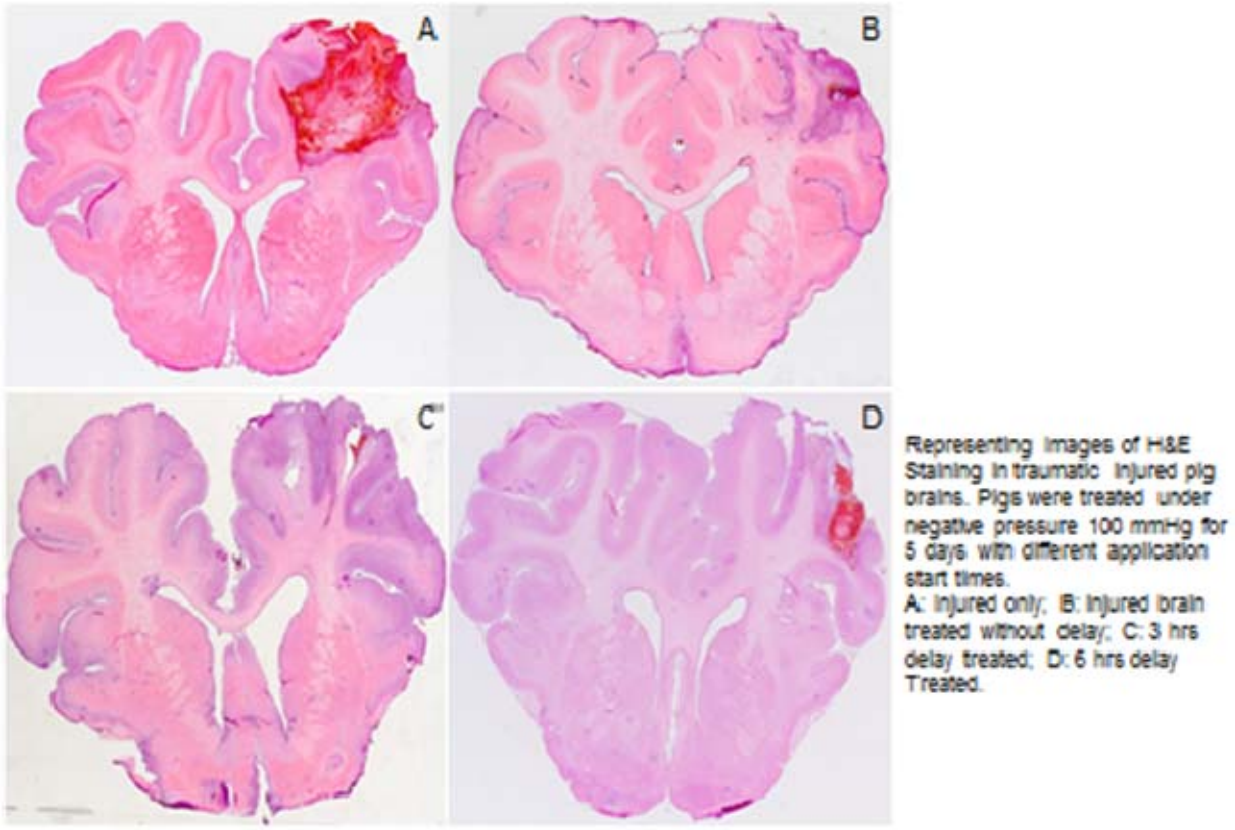


Figure 7. Representative histologic cross sections of brains for control (non-treated animals), immediate treatment, a 3 hour delay between injury creation and vacuum application, or a 6 hour delay between injury creation and vacuum application. The large area of hemorrhage and necrosis and be seen in the non-treated brain. Much smaller areas are present in the immediate (0 hour delay) and the 3 hour delay animal. A small area of hemorrhage and damage is present in the 6 hour delay brain.

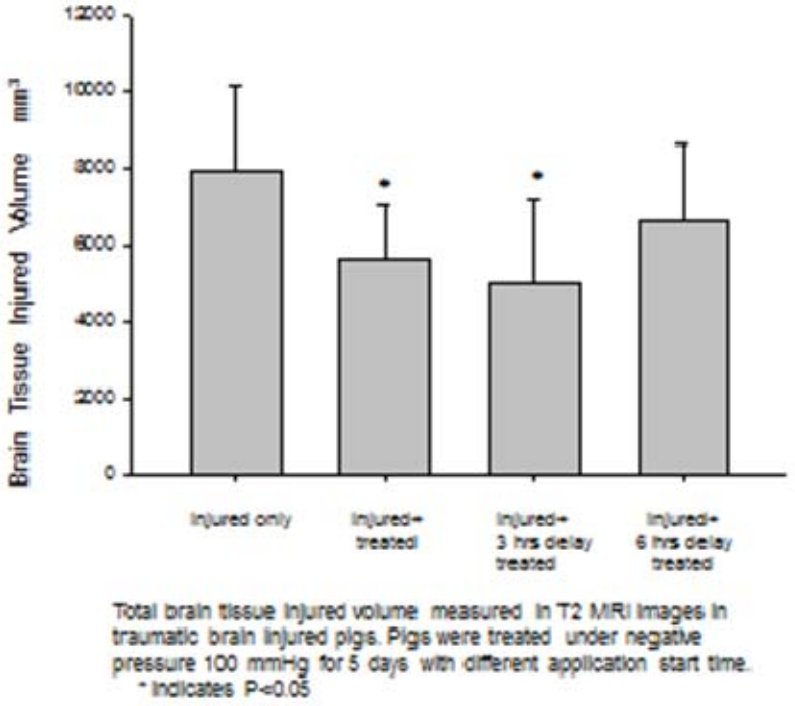
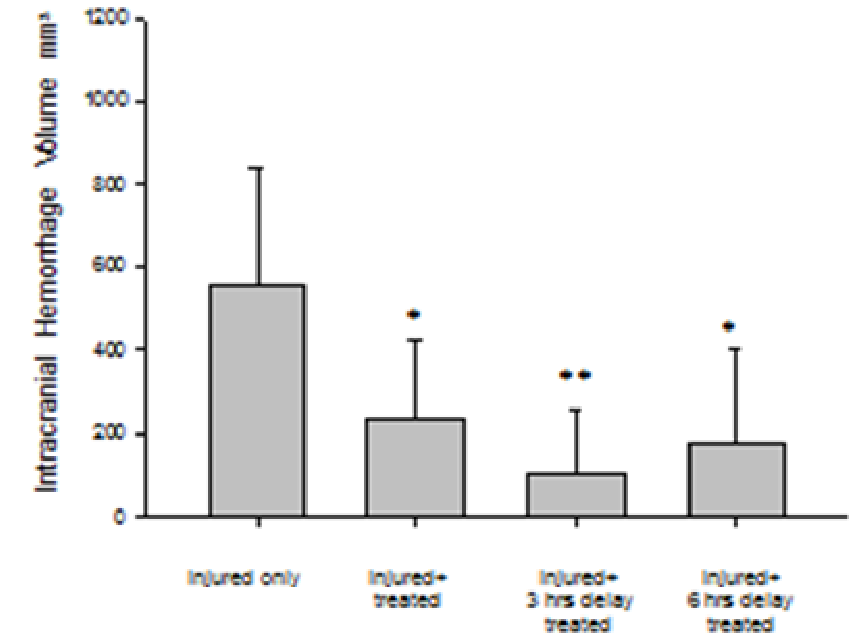


Figure 8. MRI T2 analysis of hyperintense areas of the image of the injured areas showed areas of increased water content (swelling), with largest volume in the non-treated animals. The volume of the area of injury for animals treated immediately was not significantly ($p<0.05$) different that the volume for animals treated after a 3 hour delay before vacuum initiation. The volume of the injured area for animals treated following a 6 hour delay between injury and vacuum application was not significantly smaller than the non-treated animals. The volume for the 6 hour delay animals was also not significantly larger than the areas of injury for the 0 hour or 3 hour delay animals.



Total intracranial hemorrhage volume measured in gradient echo MRI images in traumatic brain injured pigs.
Pigs were treated under negative pressure 100 mmHg for 5 days with different application start times.
* indicates P<0.05; ** indicates P<0.01

Figure 9. Gradient echo MR imaging was used to measure the volume of the hemorrhage in the animals. All treated animals had a significantly smaller volume of hemorrhage than the non-treated animals. The volume of the hemorrhage for the 0 hour delay and the 6 hour delay were similar, with the same degree of significance ($p<0.05$). The volume for the 3 hour delay was very significantly smaller ($p<0.01$) than the non-treated volume. The volumes of hemorrhage for the treated animals were not significantly different from each other.

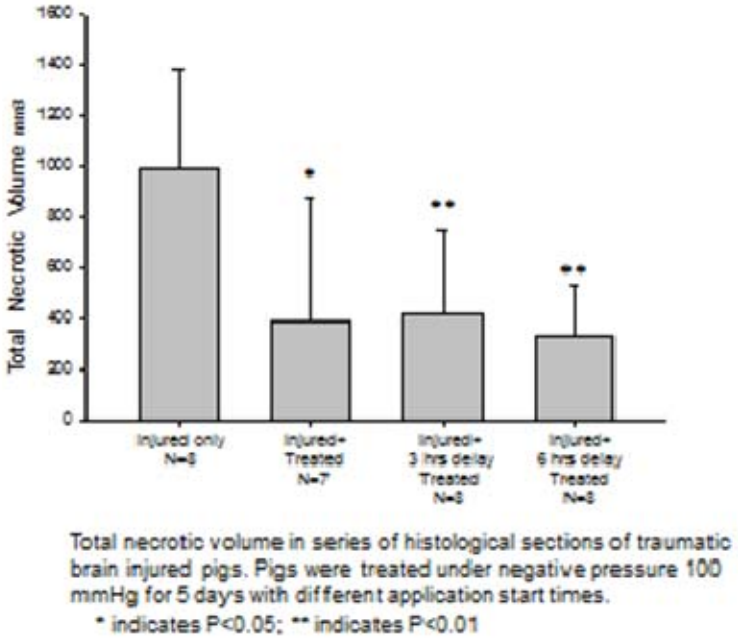


Figure 10. Histologic cross sections were imaged and the volume of the area of necrosis was determined. The volume of the area of necrosis for all treated animals was significantly ($p<0.05$) smaller than the area of necrosis for non-treated animals. The volume of area of necrosis for animals in the 3 hour and 6 hour delay groups was very significantly ($p<0.01$) than the area for the non-treated animals. The area of necrosis between the 0 hour delay and the 3 or 6 hour delay was not significantly different.

Damaged Volumes (mm3)	Injured No Treatment (Control) Mead ± SD	No Delay Treated Mean ± SD	3 hour Delay Treated Mean ± SD	6 hour Delay Treated Mean ± SD
Injured Brain Tissue Volume (T2 MRI)	7900.72±2248.78 N = 8	5621.47±1435.46 N=8	5004.30±2202.92 N=8	6919.37±2038.23 N=8
Intracranial Hemorrhage Volume (GE MRI)	553.18 ± 283.40 N=8	232.69 ± 194.47 N=8	102.94 ± 154.47 N=8	175.59 ± 229.87 N=8
Brain Necrotic Volume (H&E Staining)	958.81 ± 395.64 N=8	388.21 ± 486.26 N=7	414.91 ± 341.93 N=8	338.21 ± 195.69 N=8

Table 2. Quantitative Table with data and sample sizes for the mean damaged brain volume measurements in MR Images and H&E staining in traumatic brain injured pigs. Animals were treated with 100 mm Hg vacuum for 5 days, with varying delays between creation of injury and application of the vacuum. The sample size for histological determination of the necrotic brain volume for the 0 hour delay group was 7 animals. All other groups contained 8 animals.

Trends apparent from the MRI data show that a 3 hour delay provides for a better result than animals treated after a 6 hour delay in vacuum application. The area of injury as determined from the T2 imaging analysis exhibited a significantly smaller are of injury in animals treated after a 3 hour delay compared to animals treated after a 6 hour delay. Animals treated after a 6 hour delay still exhibited a smaller area of injury and a smaller hemorrhage volume compared to non-treated animals.

Histologic analysis of the area of necrosis shows that animals treated after the 6 hour delay had a non-statistically significant smaller area of necrosis than animals treated after a 3 hour delay. All treated animals had a statistically smaller area of necrosis than non-treated animals.

Key Research Accomplishments

Determination of application of 100 mm Hg sub-atmospheric pressure to the site of cortical injury is superior to application of 50 mm Hg and control injury

Statistically significantly smaller mean contused brain tissue volume with application of 100 mm Hg sub-atmospheric pressure compared to 50 mm Hg and control injury groups

Statistically significantly smaller mean hemorrhage volume with application of 100 mm Hg sub-atmospheric pressure compared to 50 mm Hg and control injury groups

Determination that application of 100 mm Hg sub-atmospheric pressure for 5 days to the site of cortical injury is necessary to prevent a late increase in intracranial pressure. 100% (n=10) of the animals treated for 5 days survived. 5 of 8 animals treated for 3 days died (n=3) or exhibited significant ataxia (n=2) by day 10 post injury. Treatment for 1 day was not performed due to the high mortality of animals in the 3 day treatment group.

Determination that application of 100 mm Hg sub-atmospheric pressure for 5 days to the site of cortical injury following a 3 or 6 hour delay between injury and application of the vacuum was still efficacious. Initiation of treatment after a 3 hour delay post injury resulted in as efficacious results as exhibited in animal treated immediately. Animals treated after a 6 hour delay post injury exhibited a slightly less efficacious result than animals treated immediately of following a 3 hour delay, but the treatment was still effective and significantly better than no treatment.

Reportable Outcomes

Presentations:

Zheng Z, Argenta L, Morykwas M: Mechanical Tissue Resuscitation treatment reduces injured brain tissue swelling and intracerebral hemorrhage in a pig traumatic brain injury model. 28th Annual National Neurotrauma Symposium. Las Vegas, NV. June 14-17, 2010.

Morykwas M, Zheng Z, Bryant A, Argenta L: Mechanical Tissue Resuscitation Treatment Reduces Brain Tissue Volume and Intracerebral Hemorrhage in a Pig Traumatic Brain Injury Model. 27th Army Science Conference. Orlando, FL. Nov 29- Dec 2, 2010.

Zheng Z, Argenta L, Morykwas M. Mechanical Tissue Resuscitation treatment reduces injured brain tissue swellings and intracerebral hemorrhages in a pig traumatic brain injury model (delay treatment). Congress of Neurological Surgeons 2012 Annual Meeting, Chicago, IL, October 4 - 10, 2012

Publications:

Johnston M, Zheng Z, Maldjian JA, Whitlow C, Morykwas M, Jung Y. Cerebral Blood Flow Quantification in Swine using Pseudo-Continuous Arterial Spin Labeling. (In Press) Journal of Magnetic Resonance Imaging.

Conclusion

The application of a controlled, localized vacuum to the site of a focal traumatic brain injury is able to greatly reduce several key components related to a brain injury, including mean contused volume, mean hemorrhage volume (by both histological and MRI analysis), and changes in metabolic spectral scans.

Based upon the results of this swine model (gyrencephalic brain), application of 100 mm Hg vacuum to the site of a focal TBI for 5 days was successful in greatly decreasing the size and severity of the injury. Delays of 3 hours between creation of the injury and application of the vacuum did not affect the outcomes. Delays of 6 hours between creation on the injury produced slightly less effective results, but still significantly more efficacious than no treatment.

These results offer great promise for the treatment of human focal TBI.

The expectation of rapid translation of the technique to humans is still anticipated with interest from industry for commercialization of the product and technique, although no formal licensing discussions have begun.

References

N.A.

Appendices

Personnel receiving pay from research effort:

Michael Morykwas, PhD
Louis Argenta, MD
Stephen Tatter, MD, PhD
Zhenlin Zheng, MD, PhD
Wei Du, MD
April Sprinkle Brown, BS